Delayed Increase in LDL Cholesterol Following Pentagastrin-Induced Panic Attacks

by

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Abstract

Objective: Panic disorder has been associated with an increased risk for cardiovascular morbidity and mortality. Studies have reported a correlation between increased cholesterol levels, a risk factor for cardiovascular disease, and the intensity and frequency of panic attacks. The objective of our study was to study plasma levels of low density lipoprotein cholesterol (LDL-C) in panic disorder patients and healthy controls before and after treatment with pentagastrin. **Methods:** We used a double-blind placebo-controlled crossover design with randomized injections of placebo and pentagastrin in 18 patients with panic disorder (11 men, 7 women) and 33 healthy-control subjects (24 men, 9 women). **Results:** Pentagastrin-induced panic attacks were associated with a statistically significant increase in LDL-C levels in male subjects, but not in female subjects. **Conclusion:** These findings suggest that an increase in LDL-C as a result of panic attacks may be one mechanism that may contribute to an increased cardiovascular risk in male patients with panic disorder.

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List of Abbreviations

ANOVA	Analysis of Variance
CCK	Cholecystokinin
CHD	Coronary Heart Disease
CO_2	Carbon Dioxide
CV	Cardiovascular
CVD	Cardiovascular Disease
dlPAG	Dorsolateral Periaqueductal Grey
DSM-IV TR	Diagnostic and Statistical Manual-IV Text Revised
HC	Healthy Control
5-HT	Serotonin
LDL-C	Low Density Lipoprotein Cholesterol
MAO	Monoamine Oxidase
m-CPP	meta-Chlorophenylpiperazine
MHPG	3-methoxy-4-hydroxy-phenylethylene glycol
MRS	Magnetic Resonance Spectroscopy
NaCl	Sodium Chloride
PA	Panic Attack
PD	Panic Disorder
PSS	Panic Symptom Scale
SCID DSM IV	Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders
SPECT	Single Photon Emission Computed Tomography
SSRI	Serotonin Specific Reuptake Inhibitor

THP	Tetrahydroprogesterone
VLDL	Very Low Density Lipoprotein Cholesterol

Chapter 1: Introduction

1.1 Panic disorder and cardiovascular morbidity and mortality

Panic disorder is a neuropsychiatric disorder that affects approximately two percent of the population. This disorder is characterized by recurrent unexpected episodes of severe anxiety in the form of panic attacks. The symptom cluster associated with panic attacks is outlined in the Diagnostic and Statistical Manual of Mental Disorders IV-Text Revised (DSM-IVTR), and the diagnosis of a panic attack requires at least four of the following symptoms: 1) palpitations, pounding heart, or accelerated heart rate; 2) sweating; 3) trembling or shaking; 4) sensations of shortness of breath or smothering; 5) feeling of choking; 6) chest pain or discomfort; 7) nausea or abdominal distress; 8) feeling dizzy, unsteady, lightheaded, or faint; 9) derealization or depersonalization; 10) fear of losing control or going crazy; 11) fear of dying; 12) paresthesias; 13) chills or hot flushes. Panic disorder is also characterized by a persistent concern with having another panic attack (anticipatory anxiety) or the potential consequences of the panic attack. This disorder can lead to high levels of social and occupational impairment and is also associated with an increased risk for the development of other psychiatric disorders.

Individuals with psychiatric disorders, such as panic disorder, are well known to have an increased risk of morbidity and mortality as compared with the general population. Numerous factors likely contribute to this increased mortality, including suicide, sedentary lifestyle, poor diet, substance abuse and poor access to adequate preventative health care (Ruschena et al., 1998). Epidemiological studies, however, point to a higher association between psychiatric disorders and cardiovascular disease than in the general population. This association is poorly understood, and the strength of the association varies depending on which psychiatric population is being studied.

Early investigations into the causes of an increased mortality in patients with psychiatric disorders found that panic disorder was associated with an increased risk of cardiovascular morbidity and mortality. Coryell et al. (1982) examined 113 patients with a diagnosis of panic disorder 35 years after their index admission to hospital. When they compared the findings from these patients to age- and sex-specific population figures, they found that a diagnosis of panic disorder carried an increased risk for mortality. In particular, males with a diagnosis of panic disorder carried an increased risk for death by circulatory disease. These findings were re-examined in a later study by Coryell et al. (1986), who looked at a 12-

year follow-up of 155 outpatients with a diagnosis of anxiety neurosis. They found that males with anxiety neurosis were twice as likely to die than the general population and that these excess deaths were related to cardiovascular disease and suicide. In a cross-sectional community survey that used a structured diagnostic interview for psychiatric diagnoses, but relied on patients' self reports for diagnosis of cardiovascular illness, Weissman et al. (1990) showed that panic disorder patients were at higher risk for hypertension, heart attack, and stroke compared with subjects with no psychiatric diagnoses. The relatively small number of patients with panic disorder/anxiety neurosis in the above studies and study design, however, limit the findings to that of an association between panic disorder and cardiovascular disease.

Two large prospective studies looked at the relationship between phobic anxiety, a key feature of panic disorder, and incident cardiovascular events. The first study conducted by Haines et al. (1987) as part of the Northwick Park heart study looked at the relation between scores on six subscales of the Crown-Crisp experiential index and incidence of ischemic heart disease. In 1,457 white males aged 40–64, the phobic anxiety sub-scale score was strongly related to development of major ischemic heart disease. In particular, the relative risk of fatal heart

disease in white males with a high phobic anxiety score was 3.77 as compared with white males with low scores. A similar study conducted by Kawachi et al. (1994) followed 34 000 male health professionals and measured incident cases of cardiovascular disease. The Crown-Crisp experiential index was once again used to track the relationship between anxiety and development of cardiovascular disease. Men that had high scores on the phobic anxiety sub-scale were found to have a six-fold increase in the risk of fatal myocardial infarctions. This finding remained unchanged when the investigators corrected for other known cardiovascular risk factors. Although these studies did not specifically address a population with a diagnosis of panic disorder, phobic anxiety is a key component of the diagnosis of panic disorder and raises the question if findings would be similar in a panic disorder-specific population.

Furthermore, a number of association studies have reported an increased prevalence of panic disorder in patients with coronary artery disease. In a review paper, Fleet et al. (2000) looked at studies published between 1980 and 1998 that attempted to determine the prevalence of panic disorder/panic attacks in cardiovascular disease. Eight studies were identified that used structured clinical interviews for

the diagnosis of panic disorder/panic attacks and objective measures for the diagnosis of coronary artery disease (documented history, positive stress test, or angiogram). The prevalence of panic disorder in patients with documented coronary artery disease ranged from 6.5% to 53%. This review, however, was not able to establish the directionality of the association between panic disorder and coronary artery disease. It was not clear if panic disorder leads to the development of coronary artery disease or if coronary artery disease leads to the development of panic disorder. Despite the inability to assess causality, this review highlights the high prevalence and clinical importance of panic disorder among patients with cardiovascular disease and their potential interactions.

A more recent study conducted by Gomez-Caminero et al. (2005) used a managed-care database to prospectively follow a cohort of patients diagnosed with panic disorder until their first occurrence of coronary heart disease. A total of 39,290 patients with panic disorder, and an equivalent number in a comparison group without panic disorder, were followed from initial diagnosis of panic disorder until the development of an acute myocardial infarction, unstable angina, or angina pectoris. Risk adjustments were made for age at entry into cohort, tobacco use, obesity, depression, and use of cardiovascular medications. Patients

with a diagnosis of panic disorder were found to have a nearly two-fold increased risk for the development of coronary heart disease as compared with the group without panic disorder. However, a specific etiology for this observed increase was not elucidated.

A prospective study looking specifically at the relationship between panic attacks and incident cardiovascular events was conducted as part of the Women's Health Initiative Observational Study (Smoller et al. 2007). Healthy post-menopausal women (3,369) that were living in the community and who were enrolled in the Myocardial Ischemia and Migraine Study and who had completed a panic questionnaire about the occurrence of panic attacks in the past six months were studied. Outcomes of interest in this study were cardiovascular events that occurred in women only after they had completed the panic questionnaire, and included coronary heart disease (myocardial infarction or fatal myocardial infarction), stroke, the combined end-point of coronary heart disease or stroke, and all-cause mortality. They found that full-blown panic attacks (meeting four or more panic attack symptoms) were an independent risk factor for both coronary heart disease and the combined end-point of coronary heart disease or stroke. This finding was maintained after adjustment for known cardiovascular

risk factors and depression. These findings suggest that in post-menopausal women the presence of full-blown panic attacks is an independent risk factor for coronary heart disease. This study is also of interest as most previous studies documented an increased risk of cardiovascular disease only in males with panic disorder, phobic anxiety, or anxiety neurosis.

There is, therefore, well-documented evidence of the association between anxiety and an increased risk for cardiovascular disease or death. This has been found in retrospective studies in males with a diagnosis of panic disorder/anxiety neurosis, prospective studies of males with phobic anxiety, prospective cohort studies of patients with panic disorder, and in a prospective study of post-menopausal women with a six-month history of a full-blown panic attack (Coryell et al. 1982, 1986; Haines et al. 1987; Kawachi et al. 1994; Gomez-Caminero et al. 2005; Smoller et al. 2007). Each of these studies attempted to narrow the gap between the association of anxiety and an increased risk for cardiovascular disease and a potential causal relationship. To date, however, there has not been a prospective study specifically looking at patients with a diagnosis of panic disorder and the resultant impact on risk for cardiovascular disease in this population. Likewise, there is no

clear mechanism that accounts for the cardiovascular risk incurred secondary to anxiety although studies looking at potential mechanisms have attempted to bridge the gap.

1.2 Mechanisms linking anxiety and cardiovascular morbidity and mortality

There have been several theories generated to explain the possible biological reasons for the association between panic disorder and cardiovascular disease and cardiovascular death, but the definitive mechanism(s) remains to be found (Yeragani et al., 1990; Dakak et al., 1995; Krantz et al., 1996). Although the pathophysiology of a specific panic disorder-mediated mechanism has not been elucidated, a review of the literature linking panic disorder to cardiovascular disease and cardiovascular death brings to light a number of potential mechanisms. These include decreased heart rate variability, impairments in cardiac microcirculation, and disturbances in lipid metabolism.

Basic heart rate and heart rate modulation are primarily determined by alterations in cardiac autonomic activity (Aubert and Ramaekers 1999). It is speculated that reductions in parasympathetic modulation leave the

heart exposed to uninhibited stimulation by the sympathetic system. Uninhibited sympathetic activation may result in the development of cardiac arrhythmia that can lead to sudden death. Reduced heart rate variability has been linked to an increased risk for adverse cardiovascular events including arrhythmias and sudden death in post-myocardial infarction patients (Lavoie et al. 2004). Panic disorder has also been associated with decreased heart rate variability. Studies have found evidence of reduced heart rate variability, decreased cardiac vagal tone, and elevated sympathetic activity in patients with panic-like anxiety (Kawachi et al. 1995; Piccirillo et al. 1997) and panic disorder (Yeragani et al. 1990, 1993; Klein et al. 1995; Cohen et al. 1999, 2000).

However, other more recent studies have shown that there appears to be little correlation between a diagnosis of panic disorder and decreased heart rate variability. A study by Lavoie et al. (2004) evaluated heart rate variability in 42 patients with established coronary artery disease both with (n=20) and without (n=22) panic disorder. Longer term 48-hour electrocardiographic monitoring was done to record variances in heart rate. They found that there was no clear indication of decreased heart rate variability in coronary artery disease patients with panic disorder. This study was conducted in patients with known coronary heart disease

and panic disorder whereas the initial studies were conducted in healthy patients with panic disorder. The authors discuss that the comorbid diagnosis of coronary heart disease may represent a progression of increased sympathetic activity that diminishes over time with chronic stress to the point of development of coronary heart disease. In other words, young, healthy panic disorder patients develop a parasympathetic compensation over time that decreases the impact that sympathetic over-activation has on heart rate variability. Although inconclusive, the predominant finding of cardiovascular death in patients with panic disorder suggests that a mechanism whereby cardiac rhythms are affected needs to be further explored.

Another area of investigation has been on the effects that stress has on cardiac morbidity and mortality. Like cardiac arrhythmias leading to sudden cardiac death, impaired coronary artery circulation may be another mechanism whereby panic disorder increases the risk for cardiovascular disease. Dakak et al. (1995) reported on the effects of mental stress on 10 patients with established coronary artery disease as compared with controls with normal coronary angiograms. The coronary microcirculation of patients with coronary artery disease failed to dilate during mental stress, leading to relative myocardial ischemia.

Furthermore, Fleet et al. (2005) used a 35% CO_2 panic challenge and myocardial SPECT to study the effects of panic attacks on cardiac circulation in subjects with established coronary artery disease with (n=35) and without (n=30) panic disorder. Patients needed to have had positive stress tests within two months of having the CO_2 challenge. Subjects with coronary artery disease and comorbid panic disorder were more likely to experience a panic attack in response to 35% CO_2 (74% vs. 6.7%, $p<0.001$). Subjects with panic disorder who experienced a panic attack were more likely to experience a myocardial perfusion defect than were controls who did not have a panic attack (80.9% vs. 46.4%, $p=0.009$). From these studies, it can be concluded that mental stress and panic attacks impair cardiac circulation in subjects with established coronary artery disease. It is not clear, however, if panic attacks in panic disorder patients without established coronary artery disease lead to myocardial perfusion defects and subsequent adverse coronary events.

Another potential mechanism by which panic disorder may be linked to an increased risk for the development of cardiovascular disease may be related to abnormalities in pathways of lipid metabolism. A variety of lipid abnormalities have been reported in panic disorder, and increased

cholesterol levels, particularly of low-density lipoprotein cholesterol (LDL-C), are of particular interest. LDL-C is one of the most potent cardiovascular risk factors, so much so that epidemiological data show a continuous log-linear relationship between serum cholesterol levels and risk of cardiovascular disease (Grundy et al., 2004). This means that no matter if a patient's baseline serum cholesterol level falls within a normal or abnormal range, any increase in that cholesterol level will be followed by an increase in his or her risk for cardiovascular disease (even if the increased cholesterol level remains within the normal range). Given the strong relationship between LDL-C and cardiovascular disease, understanding the role that panic disorder may have on lipid metabolism is important.

Two of the first studies to examine plasma lipid levels in panic disorder patients were conducted by Hayward et al. (1989) and Bajwa et al. (1992). Hayward et al. measured plasma lipid levels in 102 subjects diagnosed with panic disorder or agoraphobia. Their findings were that a significant number of men had cholesterol values that exceeded national reference values for cholesterol for their sex and age. Bajwa et al. set out to see if the increased cholesterol values reported in Hayward et al.'s study were attributable to the diagnosis of panic disorder or were more

generalizable to psychiatric disorders. They examined and compared the random plasma lipid levels in 30 patients with panic disorder or major depressive disorder to those in 30 control subjects. They found a more robust association of higher cholesterol levels in patients with a diagnosis of panic disorder as compared to patients with a diagnosis of major depressive disorder or the control subjects. Although these preliminary findings seemed to link increased cholesterol levels to panic disorder, subsequent studies have been inconsistent in their replication of this phenomenon.

A number of studies have compared the cholesterol levels found in panic disorder patients to a reference population with major depressive disorder and normal controls. Yamada et al. (1997) compared the total serum cholesterol levels between 46 panic disorder patients with 46 age- and sex-matched patients with major depressive disorder and with 46 age- and sex-matched patients with schizophrenia. Patients were also matched based on smoking history and alcohol consumption. Total cholesterol levels were found to be significantly higher in the panic disorder group, regardless of sex. Lacerda et al. (2000) attempted to replicate Hayward et al.'s and Bajwa et al.'s findings by measuring plasma total cholesterol levels among 85 outpatients with a diagnosis of

panic disorder (n=41), generalized anxiety disorder (n=23), or major depressive disorder (n=21). They found all patients to have a serum cholesterol level within the normal range, and no significant difference in total cholesterol levels was found between the diagnostic groups. They also reported no difference in serum cholesterol levels based on sex.

Based on the studies reported, it is not clear if there is a consistent association between the diagnosis of panic disorder and increased cholesterol levels. This, however, does not necessarily mean that the association between this psychiatric disorder and cardiovascular disease may not still be mediated in part through the dysregulation of lipid metabolism.

The studies reported above used random sampling of total cholesterol levels in patients with a diagnosis of panic disorder. The timing of cholesterol measurements may have some impact on the study findings, in particular as related to panic disorder. Two studies have suggested a correlation between cholesterol levels and the intensity and frequency of panic attacks reported by panic disorder patients (Hayward et al., 1989; Agargun et al., 1996), suggesting that elevation in cholesterol could be due to physiologic and neurochemical changes that occur during and after a panic attack. None of the previous studies, however, assessed

whether a panic attack occurred recently prior to the baseline cholesterol measurements. This failure to capture the direct temporal effects of a panic attack on cholesterol levels may have lead to the inconsistent findings of increased levels of cholesterol in panic disorder patients.

Furthermore, acute mental stress has been shown to result in increased levels of cholesterol (Muldoon et al. 1992, 1995; McCann et al. 1993; Patterson et al. 1993; Bachen et al. 2002). McCann et al. (1995) studied the effects of acute mental stress, in the form of the Stroop Colour-Word interference test and an arithmetic calculation, in healthy controls and observed an early increase in free fatty acid concentrations followed by an increase in LDL-C 24 hours after testing. Their associated findings of a similar increase of free fatty acids and LDL-C after an epinephrine infusion support a cathecolaminergic-induced release of free fatty acids and LDL-C. These and other findings (Herd 1983; Schneiderman 1983) have led to a proposed stress-induced lipolysis model in which stress-induced increases in sympathetic activity stimulate the release of free fatty acids from adipose tissue. These free fatty acids become available to the liver for the synthesis and secretion of very low-density lipoprotein (VLDL) particles that undergo modification in the circulation to become LDL particles. Kinetic studies show that the synthesis of VLDL particles

from free fatty acids and their subsequent conversion to LDL particles requires hours to days to take place (Steiner et al. 1986). Analogous to this model for psychological stress, it may be proposed that similar physiological events occur following a panic attack.

1.3 Experimental Models of Panic Disorder

In order to study the complex relationship between cardiovascular disease and panic disorder an appreciation of the pathophysiology and neurobiology of panic disorder must be examined. Experimental models designed to look at mechanisms mediating an increased cardiovascular risk in panic disorder draw on studies that make use of provocation agents to study panic attacks in laboratory settings.

Attempts have been made to study the neurobiology of panic disorder through the use of laboratory agents that pharmacologically induce a panic response akin to a spontaneous panic attack in subjects. An ideal test agent, as described in a review paper by Bourin et al. (1998), would be safe in humans, reliably reproduce the emotional and somatic symptoms that accompany panic attacks, prompt panic that patients equate to their usual panic attacks, show an enhanced sensitivity in

panic disorder patients as compared with subjects with no history of panic attacks, induce reproducible and dose related effects, and induce effects that are antagonized by known anti-panic agents. To date, there have been a number of provocation agents that have been used in research settings to induce panic and to study not only the potential activating and mediating pathways of panic, but also to study downstream neuroendocrine effects. A review of the agents used to induce a panic response and the neurotransmitter/receptor systems they impact may shed some light on the potential neurobiology of this disorder.

One of the first agents described to induce panic attacks in patients with panic disorder was sodium lactate (Pitts and McClure, 1967). The intravenous infusion of sodium lactate at concentrations of 0.5–1 M consistently induces a panic response in panic disorder patients, and to a lesser extent in healthy controls. In a study conducted by Liebowitz et al. (1985), 31 of 43 panic disorder subjects showed a panic attack in response to a lactate infusion, whereas, the 20 healthy controls showed no panic response to the sodium lactate infusion. Panic disorder patients also showed increased heart rate and evidence of hyperventilation prior to the infusion as compared with healthy controls.

Sodium lactate-induced panic attacks were also associated with biological changes associated with hyperventilation and central noradrenergic activation, but there were inconsistent findings of increased plasma cortisol and norepinephrine (Liebowitz et al. 1985). Pre-test anxiety as evidenced by increased heart rates and hyperventilation in panic disorder patients in the Liebowitz et al. study was also found to be predictive of a panic attack in response to sodium lactate in another study done by Yeragani et al. (1987) in healthy controls. Coplan et al. (1998) also found that pre-infusion levels of fear, increased cortisol levels, and decreased pCO_2 (hyperventilation) were the best predictors of a panic attack during subsequent sodium lactate infusion.

However, the precise mechanism by which sodium lactate induces panic attacks remains unclear. Activation of the ß-noradrenergic system (fight or flight) has been postulated as a potential mechanism in this model. However, failure of sodium lactate infusions to result in activation of the beta-noradrenergic system has been shown in studies using centrally acting ß-noradrenergic receptor antagonists (Munjack et al., 1989; Ravaris et al., 1991). Propranolol was not effective in blocking the panic response secondary to a sodium lactate infusion (Munjack et al., 1989;

Ravaris et al., 1991), and this finding has lead many to conclude that the principal mediating pathway of sodium lactate-induced panic is not through the ß-noradrenergic system. A theory based on a suffocation false-alarm mechanism has also been postulated (Klein, 1993). This theory postulates that hypersensitivity to perceived conditions that mimic suffocation lead to a panic response in susceptible individuals. Lactate is generated in the central nervous system in response to alterations in brain pH, most commonly alkalosis, that may be secondary to decreased CO_2 levels in the brain (as a result of hyperventilation in response to suffocation). As a result, lactate triggers a panic attack by mimicking the conditions associated with alkalosis, namely increased lactate levels. Two studies by Dager et al. (1994, 1995) using magnetic resonance spectroscopy (MRS) showed that both a 0.5 M sodium lactate infusion and hyperventilation resulted in elevated brain lactate levels in both panic disorder patients and healthy controls. Unmedicated panic disorder patients (n=3) showed elevated brain lactate levels at baseline and during and after sodium lactate infusion as compared with medicated panic disorder patients (n=4) and healthy controls (n=8). In response to hyperventilation, panic disorder patients showed a significantly greater rise in brain lactate levels as compared with healthy controls, despite similar brain lactate levels at baseline and 20 minutes

after hyperventilation. However, studies looking specifically at brain pH during sodium lactate challenges have been inconclusive. Friedman et al. (2006) used magnetic resonance spectroscopy techniques (phosphate-phosphocreatine shift) to study pH in the brain in treated panic disorder patients and healthy controls during a hyperventilation exercise. Panic disorder patients had a tendency to hyperventilate more than healthy controls, but there was no significant difference in brain pH between the two groups. This was interpreted as panic disorder patients having increased alkalotic buffering (i.e., increased baseline lactate levels in the brain) since increased hyperventilation was not associated with an increase in pH, as would be expected, compared with healthy controls with less hyperventilation. This may indicate that panic disorder patients are indeed more sensitive to subtle alterations in breathing and changes in CO_2 levels and brain pH.

Alterations in CO_2 levels constitute a separate model for panic induction in panic disorder patients. The single inhalation of air containing a level of 35% CO_2 induces panic symptoms in panic disorder patients that have been equated with endogenous panic attacks (Griez et al., 1987; Perna et al., 1994). Healthy controls appear to panic less in response to this provocation test, although the specificity of the CO_2 challenge in inducing

panic symptoms in panic disorder patients has been debated. In fact, differing percentages of panic disorder patients appear to respond to the CO_2 challenge in different studies. Nardi et al. (2006) compared the panic symptomatology of the last spontaneous panic attack between panic disorder patients who failed to respond to the CO_2 challenge test and panic disorder patients who did respond. Those panic disorder patients who did experience a panic attack post-CO_2 challenge met the criteria for the respiratory panic disorder sub-type (i.e., they experienced more respiratory symptoms during spontaneous panic attacks), had an younger age at time of diagnosis, had a stronger family history of panic disorder, and also had more previous co-morbid episodes of depression.

The specific mechanism(s) underlying the CO_2 challenge test remain unclear. Hypersensitivity of respiratory centres to CO_2 variations and brain pH in panic disorder patients may be one possibility. In essence, a similar model of the method by which sodium lactate induces panic symptoms may be invoked for CO_2. Certainly within the framework of a suffocation false alarm, increasing levels of CO_2 may be the first indication that asphyxiation may be occurring, followed by a response to hyperventilate and then flee the situation. A hypersensitivity within this

system may lead to the rapid induction of panic in susceptible patients after a single breath of 35% CO_2.

Although a fight or flight response, as panic disorder has often been characterized, is often thought of as being mediated through the sympathetic system, there are inconsistent reports of sodium lactate's and CO_2's ability to stimulate the ß-noradrenergic system. In contrast, however, the α_2-adrenergic receptor antagonist yohimbine has been shown to induce panic attacks in a significant number of panic disorder patients and to a lesser degree in healthy controls (Albus et al., 1992). Patients who experience panic attacks show downstream evidence of noradrenergic activation, displaying increased serum levels of norepinephrine's metabolite 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG) as compared with controls (Charney et al., 1992). Despite evidence to suggest involvement of noradrenergic pathways in the panic response, use of yohimbine has not led to further elucidation of potential endogenous pathways that would relate to the spontaneous generation of panic attacks in panic disorder patients.

The serotonergic (5-HT) system has been implicated in the pathophysiology of panic disorder. This has come in part from treatment

studies that have found that some pharmacological agents whose properties are mediated through serotonin, e.g., selective serotonin reuptake inhibitors (SSRIs) and the monoamine oxidase (MAO) inhibitor phenelzine, are effective anti-panic agents. meta-Chlorophenylpiperazine (mCPP) is a partial 5-HT_{2c}-receptor agonist that has been used in panic challenge studies to induce panic attacks in panic disorder patients. However, the variability in inducing a panic response in panic disorder patients and the relatively low selectivity in inducing panic attacks as compared with healthy controls has limited its utility. However, a recent study by van der Wee et al. (2004) showed that the route of m-CPP administration may have been the cause of previous inconsistencies in panic challenge studies using this agent. Van der Wee et al. found that the rapid intravenous injection of m-CPP in a dose of 0.1mg/kg over 90 seconds showed a high degree of specificity in inducing panic attacks in panic disorder patients as opposed to healthy controls. Ten panic disorder patients were compared with 10 age- and sex-matched controls. Nine of the panic disorder patients panicked in response to the rapid intravenous administration of m-CPP as compared to none of the controls. A placebo design was not utilized in this study, making it difficult to state with certainty that patients panicked in response to m-CPP versus the unfamiliar procedure and intravenous

infusion alone. However, these findings suggest that the partial 5-HT agonist m-CPP may show some promise in the future as a suitable agent to further study the mediating pathways linked to panic in panic disorder patients.

Positive allosteric modulators of the gamma-aminobutyric acid (GABA) receptor such as the benzodiazepines are also potent blockers of anxiety and panic in many psychiatric disorders. There is, however, a complex array of neurotransmitter systems that interact with the GABA system. One of the neurotransmitter systems closely linked to GABA and anxiety production is the cholecystokinin system. Cholecystokinin (CCK) is a gut-brain hormone that is found in high concentrations in the brain (Bradwejn and Koszycki, 2001). There have been two principal CCK receptor systems isolated. The peripheral CCK-alimentary (CCK-A) receptor is found primarily in the intestinal tract and digestive system, and the CCK-brain (CCK-B) receptor is found in the central nervous system. The original hypothesis for CCK's role in anxiety came from electrophysiologic studies done by Bradwejn and de Montigny (1984, 1985) that showed that benzodiazepine receptor agonists could attenuate CCK-induced excitation of rat hippocampal neurons. There have been subsequent clinical studies that have shown that two CCK-B

receptor agonists, CCK-4 and the CCK-5 analog pentagastrin, reliably and dose-dependently provoke panic attacks in panic disorder patients (Bradwejn and Koszycki, 2001).

The link between the GABA system and CCK-induced panic was further explored by Strohle et al. (2003). They measured the concentrations of progesterone metabolites, known to be neuro-modulators of the GABA system, in 10 panic disorder patients and 10 healthy controls who underwent a panic challenge using both CCK-4 and sodium lactate. These researchers found that during an induced panic attack, there were significant reductions in plasma levels of 3α,5α-tetrahydroprogesterone (THP) [allopregnanolone] and 3α,5ß-THP (pregnanolone) and significant increases in 3ß,5α-THP plasma levels. Both pregnanolone and allopregnanolone are positive allosteric modulators of GABA-A receptor function (Paul and Purdy, 1992; Rupprecht and Holsboer, 1999) and therefore potentiate GABA's inhibitory or anxiolytic function. On the other hand, the isomer, 3ß,5α-THP, is a negative allosteric modulator at the GABA-A receptor and results in a reduction in GABA-A receptor function and a decrease in inhibitory tone. Interestingly, there was no significant change in pregnanolone or allopregnanolone levels in panic disorder patients who did not panic in response to CCK-4 or sodium

lactate or in healthy controls. These findings led these researchers to believe that CCK-4- and sodium lactate-induced panic attacks may be mediated through reduction in the inhibitory tone of the GABA system. A review article by Zwanzger and Rupprecht (2005) also outlines the effect of using the anticonvulsant medications vigabatrin and tiagabine to attenuate the panic response generated by CCK-4 in healthy subjects. Vigabatrin and tiagabine both enhance endogenous GABA, by blocking GABA transaminase and inhibiting GABA transporters respectively. The ability of both of these medications to inhibit CCK-mediated anxiety in an experimental model via the GABA neurotransmitter system further strengthens the argument for a link between CCK and GABA in panic disorder.

Neuro-anatomical localization of the panic response in panic disorder patients has been difficult given the abundance and widespread nature of serotonin, cholecystokinin, and GABA within the human brain. Cholecystokinin itself has been found in high concentrations within the cerebral cortex, striatum, amygdala, hippocampus, and brainstem (Bradwejn and Koszycki, 2001). New imaging approaches such as functional magnetic resonance imaging (fMRI) (Schunck et al., 2006) are only beginning to show the promise of being specific enough to help

localize brain activity associated with panic attacks. Animal models may still prove to be useful tools in elucidating the neuro-anatomical correlates of panic. A recent study by Bertogli and Zangrossi Jr. (2005) looked at the effects of injecting CCK-4 into the dorsolateral periaqueductal grey (dlPAG) matter of rats. They found that relatively small concentrations of CCK-4 when injected into the dlPAG resulted in responses in rats akin to panic (enhanced escape behaviours from a maze). Injection of a CCK-B receptor antagonist prior to injection with CCK-4 inhibited the enhanced escape behaviour. Interestingly, localization needed to be very specific, as injections just outside the dlPAG did not elicit a response in rats. Although not conclusive, studies like these may help define target areas within the brain for further functional imaging studies in humans.

The panic reaction in patients with panic disorder is a complex neurobehavioural response that is incompletely understood. Studies with agents that stimulate a panic response in panic disorder patients in laboratory settings have allowed for the investigation of potential etiologic mechanisms and elucidation of neurotransmitter systems involved. Sodium lactate infusion and 35% CO_2 inhalation studies seem to point to dysregulation in breathing chemoreceptors and potential alterations in

brain pH. The success of treatment of panic disorder with SSRIs has lead to the use of m-CPP as a panicogenic agent. Similarly, the understanding of GABA-A receptor agonists in anxiolysis lead to the discovery of the CCK-mediated role in the panic response. Further studies are required to find a link between the pathways involved, but the use of panic inducing agents will likely continue to play a central role.

Cholecystokinin (CCK) type B receptor agonists such as CCK-tetrapeptide (CCK-4) and its synthetic analogue pentagastrin resemble Bourin et al.'s description of an ideal panic-inducing agent most closely. These two CCK-B receptor agonists have been clearly shown to induce panic attacks that are phenomenologically similar to spontaneous panic attacks experienced by patients with panic disorder (Bradwejn et al. 1991). The response to the panic-inducing effects of CCK-4 and pentagastrin is enhanced in patients with panic disorder as compared with healthy volunteers (Bradwejn et al. 1991; Van Megen et al. 1991). The panic response generated by CCK-B receptor agonists is attenuated with the use of known anti-panic agents, indicating an overlap in pathophysiological mechanisms between panic attacks in panic disorder and CCK-B receptor agonist-induced anxiety (Bradwejn and Koszycki 1994; Shlik et al. 1997; Van Megen et al. 1997). Both CCK-4 and

pentagastrin have been widely used to investigate the neurobiological changes taking place during panic attacks (Bradwejn et al. 1991; Abelson and Nesse 1994; Van Megen et al. 1996). Furthermore, unlike many panicogenic agents, CCK-4 also fulfills the criteria for a neurotransmitter, making it an in vivo candidate for anxiety genesis. As mentioned above, intravenous bolus injections of pentagastrin induce short-lived panic attacks in panic disorder patients and to a lesser degree in healthy controls (Abelson and Nesse 1994; Van Megen et al. 1996).

1.4 Objective and Hypothesis

The ability to induce panic attacks in the laboratory setting with pentagastrin permits study of the direct temporal effects of panic attacks on physiologic parameters. Physiologic parameters of importance, as demonstrated in studies outlined above, include heart rate variability, impairment in cardiac microcirculation, and alterations in lipid metabolism. However, the most potent demonstrated cardiovascular risk factor remains LDL-C. The objective of the project described in this thesis was to measure serum concentrations of LDL-C in panic disorder patients and healthy controls before and after treatment with the panicogenic drug pentagastrin. Panic symptoms were measured using

the 18-item Panic Symptom Scale. Following the model of stress-induced lipolysis, where acute stress leads to mobilization of free fatty acids from adipose tissue and subsequent conversion to LDL-C, we hypothesized that pentagastrin-induced panic attacks would be followed by a delayed increase in LDL-C serum concentrations in both healthy controls and panic disorder patients 24 hours after the induction of a complete panic attack.

Chapter 2: Material and Methods

2.1 Materials

Pentagastin was obtained from Wyeth-Ayerst, Philadelphia, PA. The intravenous catheter system (Interlink) was purchased from Baxter Healthcare Corporation, Deerfield, Ill. The laboratory screening and LDL-C analyses were conducted by the Department of Laboratory Medicine and Pathology, University of Alberta Hospital.

2.2 Subjects

Subjects were recruited for the study through local advertisement, and they received nominal compensation for their participation in the study. All the subjects underwent an initial screening visit where eligibility for the study was determined. All eligible subjects were physically healthy based on history, physical examination, laboratory screening (complete blood count and thyroid stimulating hormone level), and baseline 12-lead electrocardiogram. The diagnoses of Axis-I psychiatric disorders were established during the initial screening visit using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (SCID DSM-IV). Eligible healthy controls had no current or lifetime personal history of any Axis-I psychiatric diagnosis or a family history of

panic disorder. A diagnosis of panic disorder was made based on the DSM-IV criteria. In panic disorder patients, current and lifetime psychotic disorders, bipolar disorders, and current substance use disorders were exclusion criteria. Subjects in both groups were excluded if they smoked more than 15 cigarettes/day, drank more than five cups of coffee daily, or used illicit drugs. All subjects were medication-free throughout the study period. Panic disorder patients had to be free of psychotropic medications two months prior to the initial visit. After a complete description of the study was given to the subjects, written informed consent was obtained.

2.3 Design

In a double-blind randomized crossover design, 23 panic disorder patients (14 males, 9 females) with a primary diagnosis of panic disorder (1 male with panic disorder had concurrent major depression) and 38 healthy controls (28 males, 10 females) were randomized to receive either an intravenous injection of a five-second bolus of placebo (0.9% NaCl) or of pentagastrin (50 mcg) on two successive visits. Female subjects received their injections during the follicular phase of their menstrual cycle. Patients were required to fast 12 hours prior to each

injection visit and refrain from smoking and drinking alcohol or coffee for the 24-hour period prior to injection.

At each visit, patients were seated in a reclining chair and remained in a semi-supine position for the entire visit. An intravenous catheter was inserted in an antecubital vein 45 minutes prior to injection, and normal saline was infused at 125 ml/hour. A three-way stop valve was utilized to allow saline infusion, injection, and blood sampling from the same intravenous line. At time = 0 minutes, the intravenous line was clamped and a five-second bolus of either placebo or pentagastrin was administered, followed by unclamping of the intravenous line.

As in previous studies, the rating of panic symptoms and the occurrence of a panic attack were assessed using the 18-item Panic Symptom Scale (PSS) (Van Megen et al. 1996). The PSS was administered at t = 5 minutes post-injection. Subjects were asked to rate the severity of their symptoms on a scale of 0–4 (0 = absent, 1 = mild, 2 = moderate, 3 = severe, 4 = extremely severe). Criteria for a panic attack included the positive rating of four panic symptoms as well as a score of ≥2 on the anxiety, fear, and apprehension item. Both the patient and the rater were blinded to the injected compound.

Blood samples for measurement of LDL-C were drawn 10 minutes (t = -10 minutes) prior to the injection, as well as 24 hours post-injection under the same fasting conditions. LDL-C measurements were determined using an autoanalyzer and a UV detector (Friedewald et al. 1972).

2.4 Statistical Analysis

SPSS 13.0 for Windows was used to perform statistical analysis. An alpha level of $p<0.05$ was considered for statistical significance. The difference between baseline (t = -10 minutes) and 24-hour LDL-C, expressed as percent change in LDL-C level, both after placebo and pentagastrin injection was the main outcome variable. Repeated measures analysis of variance (ANOVA) was used with drug as the repeated measure. The difference in LDL-C was the dependent variable. Sequence, gender (male or female), diagnosis (healthy control or panic disorder), occurrence of a panic attack following pentagastrin injection (occurred or did not occur) and their interactions were the independent/predictor variables. Age was also examined as a co-variate in the repeated measures model. Backwards manual elimination was used to remove statistically non-significant main effect variables and/or

their interactions one at a time. However, biologically important variables were kept in the final model (gender, diagnosis, and occurrence of a panic attack). Individual interactions between gender and occurrence of a panic attack following pentagastrin injection for LDL-C outcome were further examined using paired t-tests within each subset of data (e.g., LDL-C of males who panicked post-pentagastrin and post-placebo, etc.). Comparisons of baseline LDL-C measurements and PSS sum intensity between diagnostic groups were compared, when appropriate, using paired or two independent samples t-test.

Chapter 3: Results

A total of 51 subjects, 35 males (24 healthy controls and 11 panic disorder) and 16 females (9 healthy controls and 7 panic disorder), completed placebo and pentagastrin injection phases with a complete data set. One male panic disorder subject completed one injection visit but could not receive the second injection because he moved. Nine subjects, 6 males (4 healthy controls and 2 panic disorder) and 3 females (1 healthy control and 2 panic disorder) were not included in the analysis because of incomplete blood samples secondary to lab error or failure to present for blood sampling 24-hours post-injection.

The panic disorder patients were older than the healthy controls [35.2 ± 12.3 vs. 25.3 ± 7.3 years old, $F(49) = 7.8$, $p<0.01$].

One subject (male with panic disorder) experienced a panic attack after both placebo and pentagastrin injections. Seventeen out of 18 panic disorder patients (94%) experienced a panic attack after pentagastrin injection as compared to 23 of 33 healthy control subjects (70%), [$\chi^2(1)=4.21$, $p=0.04$].

Table 1 presents the mean LDL-C values in both panic disorder patients and healthy controls based on gender and occurrence of a panic attack post-pentagastrin injection. As Table 2 illustrates, the interaction between drug x gender x occurrence of a panic attack was the only statistically significant effect for percent change in LDL-C levels. The order of the pentagastrin and the placebo injection did not interfere with the results and its effect was removed from the model. Age was not a significant factor when used as a co-variate in our repeated measures analysis. Figure 1 shows that males who panicked post-pentagastrin challenge showed a statistically significant greater mean individual percent increase in LDL-C after pentagastrin injection compared with the placebo injection (10.4 ± 11.3 vs. 4.3 ± 7.6, $p = 0.02$). However, Figure 2 shows that the mean percent increase in LDL-C for females who panicked post-pentagastrin was not significantly different from placebo injection (4.5 ± 4.7 and 8.4 ± 9.1, $p = 0.18$).

There was no significant difference between males vs. females and healthy controls vs. panic disorder subjects with respect to mean baseline LDL-C prior to placebo and pentagastrin injections. However, there was a trend towards a higher mean baseline LDL-C in male panic disorder patients vs. male healthy controls [2.7 ± .9 mmol/L (103.1 ± 35.5

mg/dL) vs. 2.3 ± 0.7 mmol/L (88.4 ± 28.6 mg/dL), $F(68) = 1.7$, $p = 0.07$], but that was not seen in female panic disorder patients vs. female healthy controls [2.4 ± 0.6 mmol/L (91.9 ± 23.2 mg/dL) vs. 2.3 ± 0.4 mmol/L (90.0 ± 13.5 mg/dL), $F(30) = 8.61$, $p = 0.74$]. Twenty-four hour LDL levels post-placebo injection were greater than baseline LDL levels ($p<0.001$) with a mean 6.0 ± 8.9% increase.

Panic disorder patients showed a statistically significant greater increase in PSS sum intensity after pentagastrin injection as compared with healthy controls (34.8 ± 14.0 vs. 21.6 ± 12.1, respectively, $F(49) = 0.08$, $p<0.01$). Both healthy controls and panic disorder patients displayed a greater PSS score after pentagastrin than after placebo (21.6 ± 12.1 vs. 1.0 ±1.8, $p<0.0001$ and 34.8 ± 14.0 vs. 7.6 ± 10.7, $p<0.01$, respectively). No difference in PSS scores was observed between genders.

There was no correlation found between PSS intensity and LDL-C as measured 24 hours post-pentagastrin in males who had a panic attack.

Table 1. LDL-C values in PD patients and HCs pre-/post-placebo and pre-/post-pentagastrin injections

Subjects	Mean Baseline LDL-C Placebo mmol/L	Mean 24-hr LDL-C Post-Placebo mmol/L	Mean LDL-C Difference Post-Placebo mmol/L	Mean Percent Change in LDL-C	Mean Baseline LDL-C Pentagastrin mmol/L	Mean 24-hr LDL-C Post-Pentagastrin	Mean LDL-C Difference Post-Pentagastrin	Mean Percent Change in LDL-C
Male HCs (n=24)	2.3±0.7	2.4±0.8	0.1±0.2	4.9±9.2	2.3±0.8	2.5±0.8	0.2±0.2	8.5±10.3
Male PD Patients (n=11)	2.8±1.0	2.8±0.8	0.1±0.2	4.6±8.4	2.6±0.9	2.8±0.8	0.2±0.3	10.8±13.2
Female HCs (n=9)	2.3±0.3	2.5±0.3	0.2±0.3	7.8±10.7	2.3±0.4	2.5±0.3	0.2±0.2	8.2±11.6
Female PD Patients (n=7)	2.3±0.7	2.5±0.6	0.2±0.1	9.7±6.6	2.5±0.6	2.6±0.6	0.1±0.1	5.0±4.0
Male PA Post-Pentagastrin (n=26)	2.4±0.8	2.5±0.7	0.1±0.2	4.3±7.6	2.3±0.8	2.5±0.7	0.2±0.2	10.4±11.3
Female PA Post-Pentagastrin (n=14)	2.4±0.5	2.5±0.5	0.2±0.2	8.4±9.2	2.4±0.4	2.5±0.5	0.1±0.1	4.5±4.7
Male no PA Post-Pentagastrin (n=9)	2.6±0.9	2.8±1.0	0.2±0.3	6.2±12.2	2.7±0.9	2.8±1.0	0.1±0.3	5.8±10.5
Female no PA Post-Pentagastrin (n=2)	2.1±0.3	2.3±0.1	0.2±0.2	10.2±9.0	2.2±0.7	2.6±0.5	0.4±0.2	22.9±18.1

PD Panic Disorder, *HC* Healthy Controls, *PA* Panic Attack

Table 2. Main Effects and Interactions Post-Pentagastrin Injection

Main Effect Variables	Sum of Squares	df	*F* Value	*p* value
Drug	8.79	1	0.093	0.76
Drug x Gender	202.71	1	2.13	0.15
Drug x Diagnosis	354.31	1	3.73	0.06
Drug x Occurrence of a PA	33.36	1	0.35	0.56
Drug x Gender x Occurrence of a PA	732.82	1	7.71	<0.01
Drug x Diagnosis x Occurrence of a PA	346.35	1	3.65	0.06

PA Panic Attack

Figure 1: Comparison of Mean Percent Change in LDL-C in Male Subjects after Placebo and Pentagastrin Injections

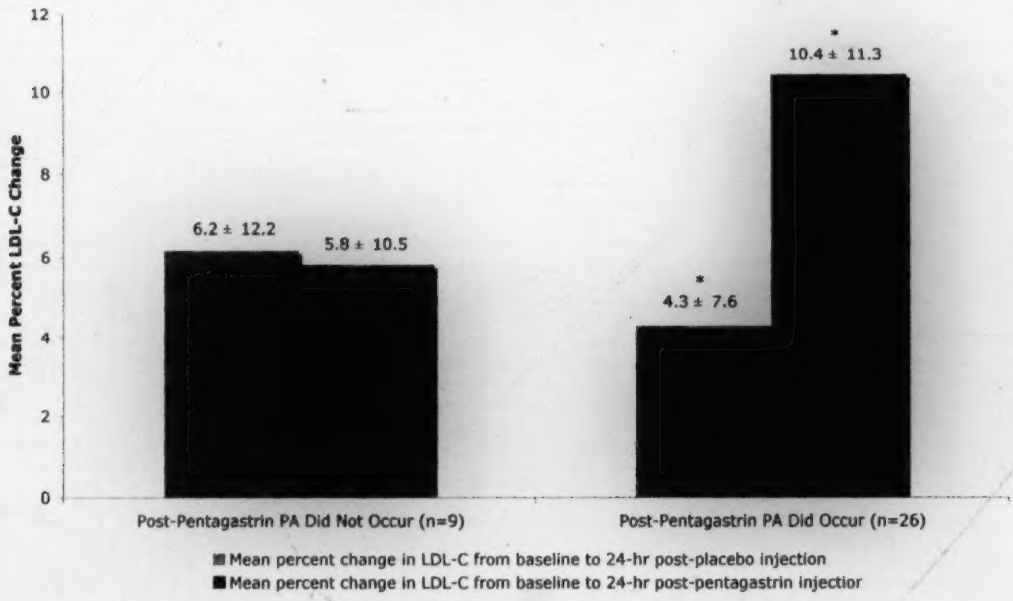
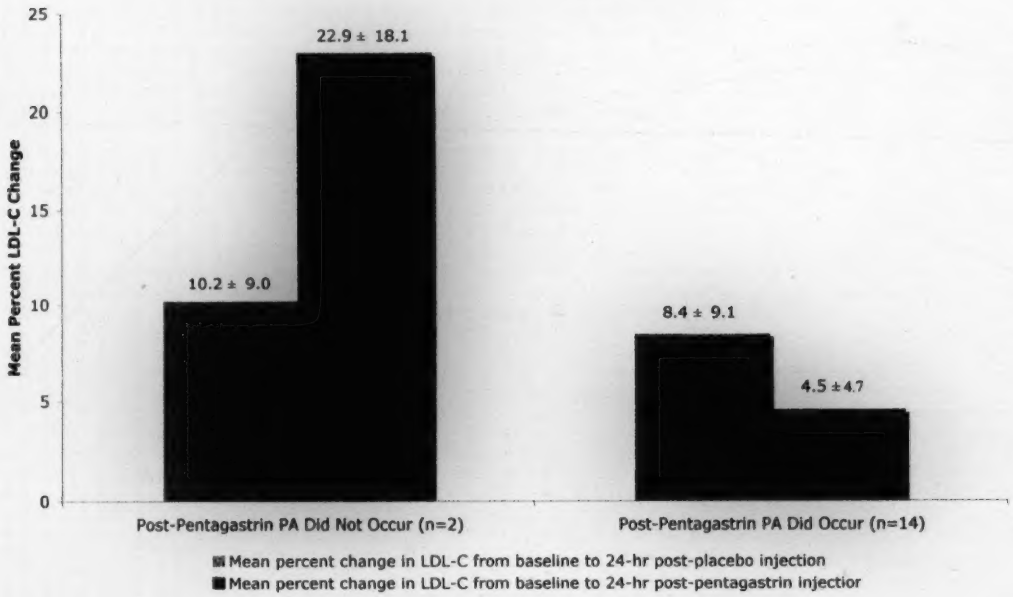


Figure 2: Comparison of Mean Percent Change in LDL-C in Female Subjects after Placebo and Pentagastrin Injections



Chapter 4: Discussion

Our results show that 24 hours after a pentagastrin-induced panic attack, plasma LDL-C concentrations are increased, as compared with placebo, in males but not females. In our study, the mean individual percent increase in LDL-C after a pentagastrin-induced panic attack in males was 10.4%. Comparatively, McCann et al. (1995) found delayed increases in LDL-C on the order of 6.2% after a mental stress test in healthy controls. The greater amplitude in LDL-C increase seen in our study could be explained by the greater inherent level of stress experienced by individuals during a panic attack.

The observed increase in LDL-C following a pentagastrin-induced panic attack was not unique to those subjects with a diagnosis of panic disorder. Subjects as a whole displayed an increase in 24-hour LDL-C after placebo injection. In our study, subjects receiving a placebo injection underwent the same procedural stress as if they were receiving pentagastrin, including venipuncture, bolus injection, and repeated blood sampling. These interventions represent a significant stress. According to the model of stress-induced lipolysis, it was expected that the placebo procedure would induce some stress in subjects and therefore result in

some increase in LDL-C. The increase in LDL-C post-pentagastrin seen in subjects who did not display a panic attack was similar to the LDL-C increase seen post-placebo injection (see Table 1). This finding argues against the increase in LDL-C post-pentagastrin being related to a pharmacodynamic effect of pentagastrin independent from a panic response. This is also supported by the lack of a general drug effect as shown in Table 2. The absolute percent difference in LDL-C attributable to experiencing a panic attack after pentagastrin as compared with placebo was on the order of 5.9% in male subjects. However, since the stress-induced increase in LDL-C is unlikely a linear relationship, it cannot be assumed that the observed 10.4% increase in LDL-C post-pentagastrin-induced panic attack is automatically the subtraction of post-placebo-LDL-C mean percent change from the mean percent change in LDL-C post-pentagastrin-induced panic attack. It is therefore most likely that the true attributable increase in LDL-C post-pentagastrin-induced panic attack lies somewhere between 5.9% and 10.4%.

In our study, there was no significant difference in the LDL-C response based on diagnosis. There was, however, a statistical trend for the interactions of drug and diagnosis and drug, diagnosis, and occurrence of a panic attack. This is most easily explained by the larger proportion

of panic disorder patients who experienced a panic attack in response to the pentagastrin infusion as compared with healthy controls, 94% and 70% respectively. The lack of a statistically significant effect is also potentially related to a lack of power in the study to detect significant interactions related to diagnosis.

As expected from previous studies (Abelson and Nesse 1994; Van Megen et al. 1996), both healthy controls and panic disorder patients showed an increase in PSS score in response to pentagastrin as compared with placebo, with panic disorder patients having a greater PSS score than healthy controls. Compared with other studies (Abelson and Nesse 1994; Van Megen et al. 1994), there was a relatively high panic rate in our study among the healthy controls, both male and female. This is likely related to the higher dosing (50 mcg) of pentagastrin than used in previous studies.

The lack of correlation between PSS intensity and 24-hour LDL-C in males is somewhat in contradiction with one report of a correlation between panic attack intensity and cholesterol levels (Agargun et al. 1996). That report described increased total cholesterol levels in panic disorder patients with sleep panic as compared with panic disorder

patients without sleep panic and healthy controls. The intensity of endogenous panic, as measured by a five-point scale in their study, is difficult to compare to PSS scores as generated by pentagastrin-induced panic symptoms. The requirement of a ≥2 score on the anxiety, fear, and apprehension item of the PSS attempts to capture the psychological distress inherent to endogenous panic attacks. The lack of correlation between PSS score and 24-hour LDL-C may also be explained by a stress-related ceiling effect that may exist in relation to panic attacks, such that further increases in panic symptomatology, after a panic attack has been triggered, do not lead to a further related increase in LDL-C above a certain threshold.

The frequency of panic attacks in panic disorder patients prior to laboratory challenge was not controlled for in our study. If an individual had experienced a recent panic attack, their LDL-C may have already been increased at the time of baseline measurement, potentially limiting the further observable increase in LDL-C secondary to a pentagastrin-induced panic attack. An alternative explanation is to consider that pentagastrin-induced panic symptomatology may be secondary to a pharmacological effect of pentagastrin. Therefore, these pharmacologically induced physical symptoms would also be measured

with the PSS but would not necessarily relate to the emotional distress associated with the panic symptoms. This view is supported by recent data (Abelson et al. 2005) showing that a cognitive intervention prior to pentagastrin infusion decreased the emotional response to pentagastrin but not the physical response in panic disorder patients. These authors suggest that the panic response to pentagastrin is better represented by the secondary emotional distress rather than the physical panic response. It is therefore conceivable that subjects with the highest emotional response, represented by those subjects who panicked post-pentagastrin, are the ones with the highest autonomic reactivity, and therefore are more likely to display an increase in 24-hour LDL-C. As a result of this conceptualization of pentagastrin-induced panic attacks, the lack of correlation between the PSS score and the 24-hour mean percent change in LDL-C would not be unexpected since the physical panic response to pentagastrin receives preferential weighting on the PSS.

The lack of a panic attack-induced increase in LDL-C levels in female panic disorder patients is interesting in the context of findings of increased cardiovascular mortality in male panic disorder but not female panic disorder patients in a study that did not control for cholesterol levels (Coryell et al. 1986). The observed gender difference in our study

cannot be attributed to menstrual cycle-related hormonal fluctuations considering that the injections in female subjects were performed during the early follicular phase when concentrations of female hormones are at their nadir. It is unlikely that this gender difference is explained by an attenuating effect of estrogens on the release of free fatty acids since we have shown that pre-treatment with ethinyl estradiol did not affect the release of free fatty acids (Morrow et al. 2003) following pentagastrin-induced panic attacks. However that study was only performed in males. The more recent findings of an increased cardiovascular morbidity and mortality in post-menopausal women associated with a six-month history of a panic attack, however, suggest a more complex relationship between panic anxiety and hormonal status (Smoller et al. 2007). The lack of an observed increase of LDL-C in female subjects in our study may also be secondary to the relatively small sample size of 16 females, 9 of whom were panic disorder patients. Furthermore, the fact that only 2 female subjects did not experience a pentagastrin-induced panic attack limits the ability to compare between group differences.

Although a mechanistic hypothesis was not tested in this study, there is evidence from previous research that suggests panic attack-induced LDL-C increases in male subjects mimic the model of stress-induced

lipolysis. It has been reported that pre-treatment with an infusion of propanolol, a non-specific ß-adrenergic receptor blocker, dramatically attenuated the behavioural and cardiovascular response to CCK-4 in male healthy controls (LeMelledo et al. 1998). Khan et al. (2004) reported a similar observation in an experiment using pentagastrin as a panicogenic agent. Interestingly, that research group found that propranolol pre-treatment resulted in a significant decrease in subjective reports of anxious distress only among male healthy controls, but not female healthy controls. These two studies suggest that there may be a gender difference in the sympathetically mediated anxiety/panic response to CCK-B receptor agonists. The anxiety and panic response in females may be mediated to a lesser degree through the sympathetic system as shown by a differential response to pre-treatment with a ß-adrenergic receptor antagonist. A difference in sympathetic activation between males and females in the panic response would result in a difference in LDL-C response according to the proposed model for stress-induced lipolysis, as observed in our study. One study reported increased baseline LDL-C in panic disorder females, according to now obsolete normative reference ranges, but in fact, in that study, male and female panic disorder patients had equal absolute levels of LDL-C (Hayward et al. 1989). Furthermore, the study did not control for

fluctuations in LDL-C as a result of the normal menstrual cycle (Barnett et al. 2004). These observations suggest that CCK-B-induced panic symptoms are at least partially mediated by the sympathetic system in males.

In addition to sympathetic activation, our laboratory has also shown that pentagastrin-induced panic symptoms are followed by a dramatic increase in plasma levels of free fatty acids two hours after induction of panic symptoms in males (Morrow et al. 2003). Therefore, our current observations of increased LDL-C levels 24 hours post-panic attack lend further support to a stress-mediated pathway resulting in downstream increases in cholesterol levels.

Our findings suggest a temporal relationship between a panic attack and subsequent increases in LDL-C in males. Previous studies on baseline LDL-C and panic disorder have not taken into account the proximity of the most recent panic attack. Individuals with infrequent panic attacks may not demonstrate an increase in LDL-C if measurements are obtained at a time too long following a panic attack. This source of heterogeneity may lead to inconsistent reports of increased LDL-C in panic disorder patients in general. This concept is supported by previous

studies that have shown a correlation between the frequency and intensity of panic attacks and increases in LDL-C (Hayward et al. 1989; Agargun et al. 1996). The present findings of a gender effect may further account for the inconsistency in studies that included both males and females in their studied population.

Elevated serum cholesterol is causally associated with increased risk of coronary heart disease. Optimal LDL-C levels are based on age, the presence of cardiovascular risk factors, and the calculation of a 10-year risk of coronary heart disease-related death or a non-fatal myocardial infarction (Grundy et al. 2004). Guidelines for lipid management are based on the demonstrated reduction of major adverse cardiac events in both primary and secondary prevention trials (Grundy et al. 2004). The most recent National Cholesterol Education Program (NCEP) guidelines' target levels for LDL-C are <4.14 mmol/L (160 mg/dL) for low-risk patients, <3.37 mmol/L (130 mg/dL) for moderate-risk patients, and < 2.59 mmol/L (100 mg/dL) for high-risk patients. High-risk patients include patients with coronary heart disease and coronary heart disease risk equivalents such as diabetes and an LDL-C >2.59 mmol/L. These patients have a greater than 20% chance of myocardial infarction or cardiac death within 10 years (Grundy et al. 2004). Data from recent

clinical trials with cholesterol-lowering agents demonstrate that, regardless of baseline measurements, reduction in LDL-C levels of between 30 to 40% in high- and moderately high-risk patients correlate to a similar percentage decrease in cardiovascular death risk (Grundy et al. 2004).

Any LDL-C above 2.59 mmol/L (100 mg/dL) is atherogenic, even in healthy subjects (Grundy et al. 2004). It is not unusual for untreated panic disorder patients to experience panic attacks on a daily basis. Based on our results of a 10.43% increase in LDL-C induced by the occurrence of a panic attack, we can assume that a significant number of panic disorder patients may present with a chronic increase in LDL-C of at least 10% associated with the occurrence of their panic attacks. Interestingly, in this study's relatively small sample, without controlling for the proximity of the most recent panic attack, demonstrated a statistical trend towards increased baseline LDL-C levels in the male panic disorder patients as compared to healthy controls. A 10% increase in serum cholesterol is associated with a 20 to 30% increase in risk for coronary heart disease, and elevations earlier in life may be associated with higher increases in risk (Gaziano et al. 2005). As mentioned above, any LDL-C above 2.59 mmol/L (100 mg/dL) even in healthy subjects

carries an associated increase in coronary heart disease risk. Interestingly, in our physically healthy group of panic disorder patients, 64% of the male panic disorder patients had LDL-C levels above 2.59 mmol/L (100 mg/dL) post-pentagastrin-induced panic attack. According to the NCEP guidelines, however, therapeutic management of LDL-C levels, as seen in our study, would not be required in these physically healthy panic disorder patients. Given the high prevalence of panic disorder in patients with coronary heart disease, the potential clinical relevance of our findings is further strengthened (Fleet et al. 2000).

A 10% cumulative increase in LDL-C as seen in our study would, if chronic, therefore be associated with an increased risk for coronary heart disease. The increase in LDL-C noted in our study reflects a one-time measurement of cholesterol 24-hours after stress induction and a pentagastrin-induced panic attack. This increase may under- or over-represent what occurs in panic disorder patients when they experience spontaneous panic attacks. Despite this, patients with panic disorder experience anxiety related not only to panic attacks, but also in relation to the anticipation of having a panic attack. This may lead to levels of basal stress akin to acute mental stress, already shown to be linked to increases in free fatty acids and LDL-C. Therefore, a combination of

increased basal stress, punctuated by panic attacks, may lead to a chronically increased baseline LDL-C in panic disorder patients and therefore, in part, mediate increased cardiovascular risk.

No prospective studies linking panic disorder and coronary heart disease have yet been published. However, prospective studies have linked phobic anxiety, a key feature of panic disorder, to an increased risk for cardiovascular mortality in males (Haines et al. 1987; Kawachi et al. 1994). Smoller et al. (2007) also found an increased risk for cardiovascular morbidity and mortality in older women who had experienced a panic attack in the six months preceding a cardiovascular event. The results of the two larger studies (Kawachi et al. 1994; Smoller et al. 2007) were presented after correction for known confounding factors such as LDL-C. Correcting for LDL-C in a prospective study, based on our findings, may negate the impact of a major contributor to the increased cardiovascular disease risk noted in panic disorder patients. Future prospective studies on the impact of panic disorder on cardiovascular disease should present data prior to and after correction for LDL-C levels in order to assess the specific contribution that elevated cholesterol levels may have in mediating cardiovascular risk in panic disorder patients. Ideally, such prospective

studies would also include other factors suspected of playing a role in the increased cardiovascular risk in panic disorder patients; for example, decreased heart rate variability and impaired cardiac microcirculation (Yeragani et al. 1990; Dakak et al. 1995; Krantz et al. 1996).

Ideally, our results should be confirmed by an investigation of the effect of a spontaneous panic attack on LDL-C. Although our findings suggest the observed panic-attack-induced increase in LDL-C was not related to a pharmacodynamic effect of pentagastrin, there may still be subtle differences between spontaneous and laboratory-induced panic attacks. Also, we measured LDL-C levels only once 24 hours post-pentagastrin-induced panic attacks and cannot, therefore, make any inferences as to the duration of LDL-C elevation post-panic attack. The timing of our LDL-C measurement was based on the findings by McCann et al. (1995) that LDL-C was elevated 24 hours after acute mental stress. The time specific effect of panic attacks on lipid metabolism is not clear from our study, and a chronic elevation of LDL-C in a panic disorder patient with infrequent panic attacks may be an overestimation.

Although a tempting speculation, it remains to be determined if successful anti-panic treatment, resulting in a decreased frequency and

intensity of panic attacks, would result in decreased LDL-C levels in panic disorder patients. This is worth addressing, particularly in light of recent findings of increased LDL-C levels induced by SSRIs and combined serotonin and norepinephrine reuptake inhibitors (SNRIs) which are first line drugs used in treatment of panic disorder (Lara et al. 2003; Rickels et al. 2004; Liebowitz et al. 2005).

Chapter 5: Conclusion

In summary, we found an increase in LDL-C levels 24 hours following a pentagastrin-induced panic attack in males. These results suggest that the proximity of a panic attack to the timing of LDL-C measurement may be key in interpreting an LDL-C increase in panic disorder patients. These results would indicate that male patients with panic disorder may, as a result of frequent panic attacks, have chronically increased baseline levels of LDL-C, which could at least partially mediate the increased cardiovascular risk in panic disorder patients. Such awareness would be particularly relevant in the numerous panic disorder patients who also have co-morbid cardiovascular illness, as current cardiovascular guidelines recommend aggressive LDL-C lowering treatment in patients with established coronary heart disease (Grundy et al. 2004).

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